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CREPIS NICAEENSIS Balb. × C. SETOSA Hall. f.

MOST CREPIS SPECIES are excellent subjects for cytological research because of their generally large and morphologically distinct chromosomes. The genus includes three subgenera, in each of which numerous representative species have been studied with reference to comparative morphology, geographic distribution, and chromosome relations (Babcock and Cameron, 1934; Babcock, 1936). Cytogenetic studies on several hybrids between species in the same subgenus and in different subgenera have exhibited phenomena that throw light on problems of taxonomy, phylogeny, and evolution (Babcock and Navashin, 1930). The present investigation was undertaken with the hope of contributing further evidence on some of these problems.

SPECIES AND HYBRIDS INVESTIGATED

These investigations are concerned with hybrids between *Crepis nicaeensis* Balb., of subgenus *Eucrepis,* and two subspecies of *Crepis setosa* Hall. f. of subgenus *Barkhausia.* Some of the F_2 derivatives were also studied. Plants of the two subspecies of *C. setosa,* namely, *typica* (accession 2623) and *Topaliana* (accession 2671) were used as paternal parents, and a single *nicaeensis* plant (accession 2700) furnished the maternal gametes. The diploid chromosome number of both species is 8.

The strain of *C. nicaeensis* used in this study came from northern Italy. The species occurs sporadically in lower montane regions from the Eastern Pyrenees to Macedonia. Plants found in the wild state are tall, erect, and simple-stemmed with a few branches near the top bearing a few medium-sized flower heads. Under cultivation the plants are either annual or biennial; they retain the same upright habit as in the wild but are very vigorous, with long branches and many heads. As a rule *nicaeensis* plants have a stronger central axis than do *setosa* plants and the basal or rosette leaves are very different, although there is noticeable variation in both species. In details of the heads, florets, and fruits the differences are even more marked. Differences in the achenes

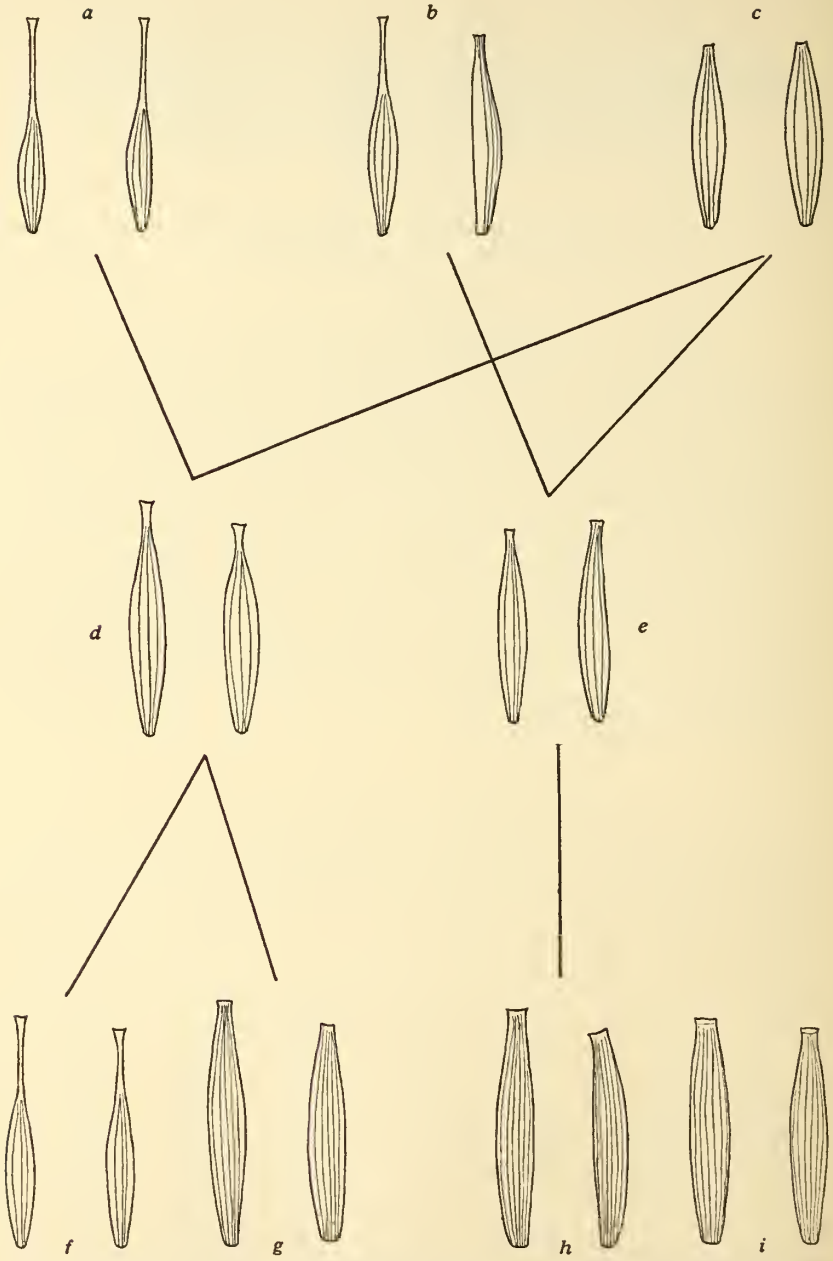

Fig. 1. Achenes of: *a, Crepis setosa typica* (2623); *b, C. setosa Topaliana* (2671); *c, C. nicaeensis* (2700); *d, C. nicaeensis* × *C. setosa typica* F_1; *e, C. nicaeensis* × *C. setosa Topaliana* F_1; *f*, an F_2 derivative with 8 chromosomes; *g, h*, F_2 derivatives with 24 chromosomes; *i, C. biennis.*

are of special interest in this study. In *nicaeensis* the achenes are golden brown, about 3 mm. long and 0.6 mm. wide, terete, narrowed at base and summit, with no beak or prolongation of the apex, and with ten broad, nearly smooth, longitudinal ribs (fig. 1*c*).

In *C. setosa,* considered as a whole, the plants are more slender, the habit more bushy, and the heads more numerous and smaller. Besides other distinguishing characters, the achenes are tawny, 3–5 mm. long and 0.3–0.6 mm. wide, and the body is terete, with ten ribs, narrowed at base and attenuate at summit into a slender beak equal to or shorter than the body (fig. 1*a, b*). The strain of *C. setosa typica* used in these investigations was obtained from a wild population in Savoy, France. This subspecies occurs rather commonly at low elevations from the eastern Pyrenees to Macedonia. Our accession of subsp. *Topaliana* came from a wild population in eastern Thessaly. This subspecies has a restricted distribution in northern Greece and a few forms which are intermediate between the two subspecies have been found in northwestern Thessaly and Epirus.

Some of the characters that distinguish the two subspecies are listed in table 1. Although these data were obtained from only two plants of one subspecies and one of the other, yet these plants were fairly typical of their respective entities. Plants of *typica* are normally larger and more robust than *Topaliana* plants. Differences in the basal and cauline leaves are shown in figure 2*a, b*. Certain differences in the achenes are discernible in figure 1*a, b*. In the *typica* strain the beak is equal in length to the body of the achene and all the achenes in a head are closely similar. In *Topaliana* the marginal achenes are notably different from the rest of the achenes in the head. They are somewhat compressed, paler in color, and merely attenuate, not finely beaked, as is shown by the right-hand achene in figure 1*b,* and the inner achenes have a relatively longer body and shorter beak than do those in the *typica* strain used. Another difference which, although minute, was consistent in each plant was found in the setae (bristles) borne on the involucral bracts and peduncle. In the *typica* strain these setae are slightly longer and more slender than those of the *Topaliana* strain. This is especially interesting because of the failure of setae to appear in the F_1 (30.31) of which *typica* was a parent, whereas setae were abundant in the other F_1 (30.32), as is shown in plate 1*d, e*. The first impression given by these facts is that the particular *typica* plant which was used as male parent of 30.31 must have been heterozygous for setae, whereas the *Topaliana* plant used in producing 30.32 was homozygous. It was shown by Collins (1924) that in *Crepis capillaris* glabrous involucre it inherited as a simple recessive to pubescent involucre. But here the pubescence consists of hairs, not setae. Furthermore, plants with glabrous involucres have never been reported

in *C. setosa;* on the contrary, these peculiar setae seem to be a constant specific character in this species. Certainly no plants with glabrous involucre were observed among several strains of *typica* while they were under cultivation. This fact, together with the structural difference in the setae of the two subspecies noted above, may be indicative of a

TABLE 1

Differentiating Characters in the Subspecies of *Crepis setosa*

2623. Typica	*2671. Topaliana*
Large, robust	Small, slender
Height (2 plants), 65-75 cm.	Height (1 plant), 38 cm.
Stem erect with several long branches from base, each with a secondary branch from each node; secondary branches erect, forming a narrow angle with main branch	Stem erect with a branch from each node, but branches shorter than axis and spreading at a wide angle from it
Involucre setose, the bristles slightly longer and more slender than in 2671. Bristles of same type also rather plentiful on peduncle	Involucre setose, the bristles shorter and slightly thicker. Bristles on peduncle very few and diminished
Achenes all similar, or the marginal ones parthenocarpic, but even then usually beaked and colored like the inner ones; 3-4 mm. long, deep tawny, the body oblong, subterete, strongly 10-ribbed, beak equal to body, conspicuously expanded to form a white inverted cone below the broad pappus disk	Achenes of two shapes, 3-4 mm. long; the marginal ones very pale or whitish on ventral face, long-fusiform, strongly attenuate toward summit or shortly beaked, obscurely ribbed; inner achenes pale tawny, the body fusiform, subterete, delicately 10-ribbed, beak a little shorter than body, not expanded below pappus disk
Style branches bright green	Style branches pale greenish yellow

genetic difference between the subspecies with respect to setae. Attempts were made to obtain hybrids between these two subspecies of *C. setosa,* but they failed, so there is no further evidence bearing on this particular question.

Hybrids between *C. nicaeensis* and *C. setosa* were obtained with difficulty. Only four F_1 plants were produced, two of each with *typica* and *Topaliana* as male parent. For brevity's sake the two crosses will be referred to hereafter as hybrid A and hybrid B. The F_1 hybrid sibs were very similar, but there were consistent differences between the two F_1

families. Some of these differences are shown in table 2. The leaf types of the parents and F_1 hybrids are shown in figure 2. Not much significance can be attached to these differences because the parents, like those in other species of *Crepis,* are rather variable in leaf shape; but, taken along with the other differences in the hybrids, these leaf differences may also indicate genetic diversity between the subspecies. Certain differences in anthocyanin in the F_1 plants may have been due to heterozygosity in the *nicaeensis* parent. One plant from each cross was pressed

TABLE 2

DIFFERENTIATING CHARACTERS IN HYBRIDS A AND B (2 PLANTS OF EACH)

A. *C. nicaeensis* ♀ × *C. setosa typica* ♂	B. *C. nicaeensis* ♀ × *C. setosa Topaliana* ♂
Achenes average 4.27 mm. long; ribs as in *C. nicaeensis*	Achenes average 3.47 mm. long; ribs similar but less prominent
Involucres and peduncles glabrous	Involucres and peduncles setose
Anthocyanin restricted to base of plant	Anthocyanin present in upper part of plant
Basal and cauline leaves laciniate	Basal leaves coarsely dentate; cauline leaves nearly entire

for the herbarium; the others were used in the present study. The two hybrids differed notably in fertility. Hybrid A produced 545 open-pollinated achenes and hybrid B, only 120.

From hybrid A one hundred F_2 achenes were sown. They produced ninety-three seedlings. Three of these died at the cotyledon stage and three more after they had developed a small rosette of basal leaves (see plate 2*h, i*). The chromosomes of the six seedlings that died were not examined. The remaining plants were grown to the large rosette stage. Chromosome numbers were determined from somatic plates in root tips. Only three had 8 chromosomes; the remaining eighty-four all had 24 chromosomes.

From hybrid B twenty-five achenes were sown, and nineteen seedlings were obtained. Two of these died in the cotyledon stage. The other seventeen were grown to maturity; of these, two had 8 chromosomes and fifteen had 24 chromosomes.

The 24-chromosome F_2 derivatives must have resulted from natural crossing with *Crepis biennis* ($n = 20$). Plants of this species were in flower along with the F_1 hybrids, which were purposely left unprotected. The leaves of the 24-chromosome derivatives were more or less like those

of *C. biennis*. This is not necessarily significant, because the leaves of *nicaeensis* also resemble those of biennis; but when these leaves are compared with those of their 8-chromosome sibs, the difference is striking

Fig. 2. Basal and cauline leaves from: *a, Crepis setosa typica; b, C. setosa Topaliana; c, C. nicaeensis; d, C. nicaeensis* ♀ × *C. setosa typica* ♂ × F_1; *e, C. nicaeensis* ♀ × *C. setosa Topaliana* ♂ F_1.

because the leaves of the latter strongly resembled those of *setosa* (see plate 15*f, g*), whereas the leaves of the 24-chromosome plants were very like those of *biennis* (plate 15*a–e*). Fortunately these 24-chromosome derivatives were slightly fertile, and the achenes were like those of *bien-*

nis in size and shape (see fig. 1) and in the higher number of ribs which is characteristic of *biennis*.

The five 8-chromosome F_2 derivatives were closely similar in general appearance and very precocious in development as compared with the 24-chromosome sibs. Two of them are shown in rosette stage in plate 15*f, g*. The one on the right is three weeks older than the other. Two of the 8-chromosome derivatives died from an unknown cause when the flower stalk was a few inches high, and another was attacked by a species of *Botrytis* just before flowering. The two plants that produced flowers were from hybrid A. Their fertility was low, one producing twelve achenes and the other, seven.

This paper is chiefly concerned with a study of meiosis in the F_1 hybrids and the two 8-chromosome F_2 derivatives that produced flowers.

Cytological Methods

Root tips were fixed in chromacetic formalin and stained with Haidenhain's iron haematoxylin, as described by Hollingshead and Babcock (1930). At first the buds were fixed in the Carnoy-Navashin solution, dehydrated in the alcohols, cleared in xylol, and imbedded in paraffin. Buds treated in this manner were usually cut with difficulty because of hardening in the higher alcohols. To remedy this situation, a butyl alcohol series was substituted as a clearing agent in place of the regular xylol series. In this method the buds were run up to 80 per cent alcohol, then through the following series of alcohols (Zirkle, 1931) :

(*a*) Water 15 cc., absolute ethyl alcohol 50 cc., butyl alcohol 35 cc., 2 to 3 hours.

(*b*) Water 5 cc., absolute ethyl alcohol 40 cc., butyl alcohol 55 cc., 2 to 3 hours.

(*c*) Absolute ethyl alcohol 25 cc., butyl alcohol 75 cc., 2 to 3 hours.

(*d*) Butyl alcohol. At least 2 changes, leaving in the 2d change at least 12 hours.

Infiltration was accomplished by pouring melted paraffin into a vial, allowing it to cool, and then pouring over it butyl alcohol containing the buds. As few as four changes in the oven over a period of two days were found to be sufficient to remove all traces of butyl alcohol; the buds treated in this manner were soft, easily cut, and stained readily in both gentian violet and Haidenhain's iron-alum haemotoxylin.

In order to concentrate more material on a slide, imbedded buds were remelted in the oven, and from two to four were mounted on one block. Melted paraffin was slowly dropped over the buds and cooled rapidly, and the block was finally trimmed for cutting. Both cross and longitudinal sections were cut. The latter proved to be most useful, because a large number of PMC's could be studied in the same locule, and because irregular stages found in F_2 plants were more easily studied when ad-

jacent to "normal" cells. All slides from each block were kept separate, and a slide containing material from near the center of the buds was stained to determine whether the desired meiotic figures were present; if so, the remaining slides of the series were stained.

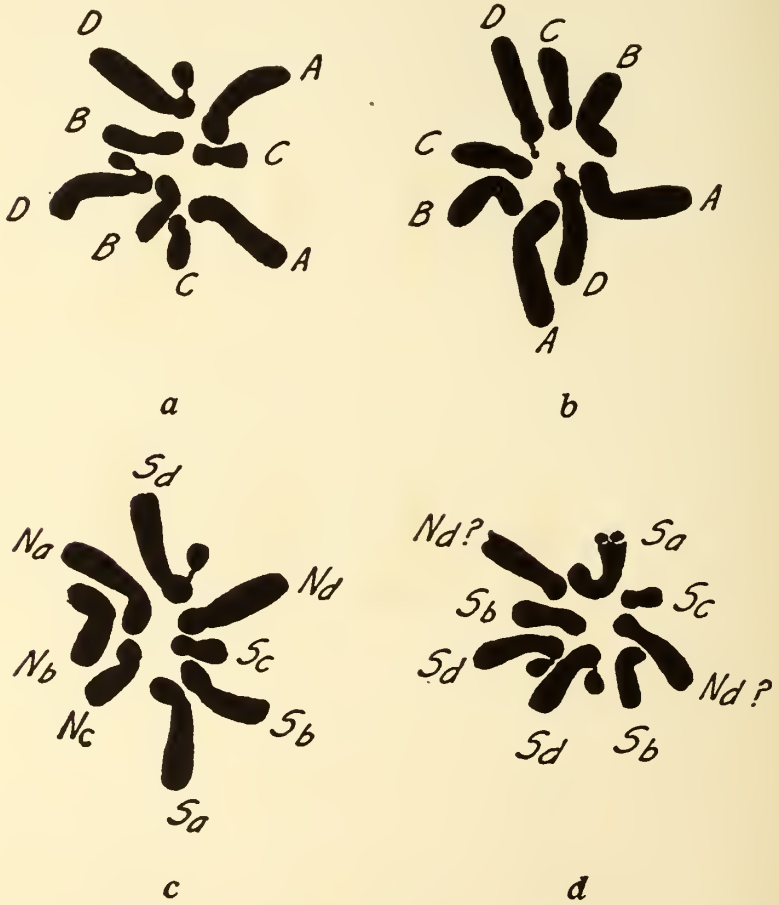

Fig. 3. Chromosomes at mitotic metaphase in root-tip cells of: *a*, *Crepis setosa*; *b*, *C. nicaeensis*; *c*, *C. nicaeensis* × *C. setosa* F_1; *d*, an 8-chromosome F_2 derivative. × 2500.

Figure 3 and figure 4*c* were drawn at a magnification of 2500 diameters and were reproduced without reduction. Figures 4*a* and 4*b* were drawn at a magnification of 4000 diameters and reproduced without reduction. Figures 5*e* and 6*c* were drawn at a magnification of 1800 diameters. All other drawings were made at a magnification of 2500 diameters and were reduced one-fourth in reproduction.

Somatic Chromosomes

Somatic metaphase chromosomes of *Crepis setosa* are shown in figure 3*a;* those of *C. nicaeensis* in figure 3*b*. As the chromosomes of the two *setosa* subspecies were apparently identical, only one plate is shown. According to Navashin's scheme of chromosome designation, both species have one pair each of types A, B, C, and D. These designations are not

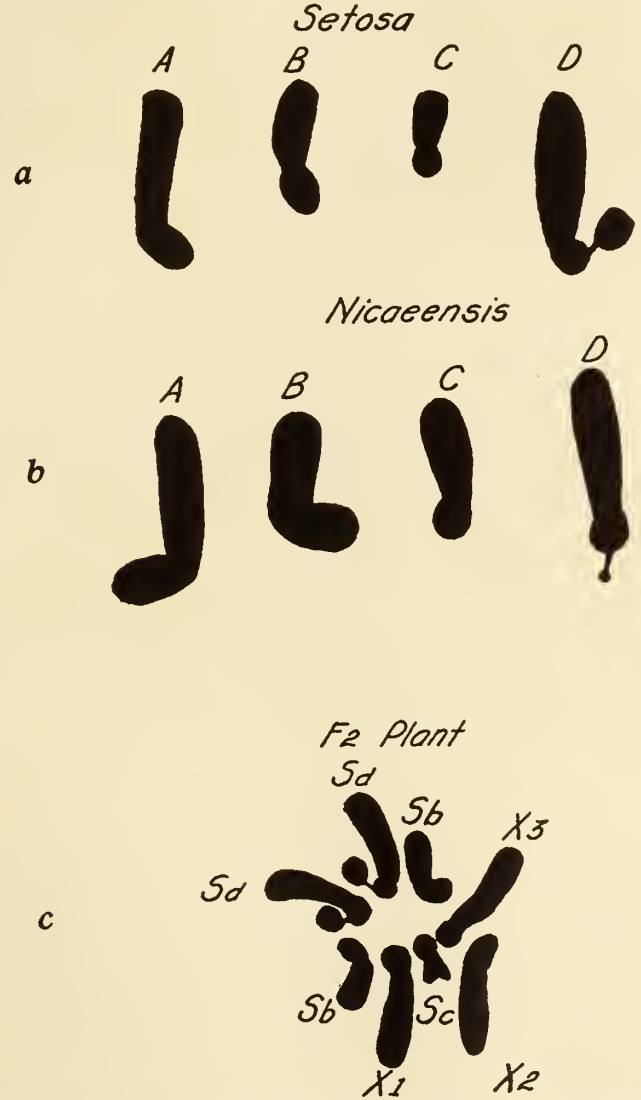

Fig. 4. *a, b,* Haploid chromosomes of *Crepis setosa* and *C. nicaeensis* × 4000. *c,* chromosomes at mitotic metaphase in a root-tip cell of an 8-chromosome F_2 plant showing three doubtful members, X_1, X_2, X_3. × 2500.

intended to imply homology but merely general similarity in size and shape. There is difference enough in chromosome morphology to make identification of each set in F_1 a relatively simple matter (fig. 3*c*). Navashin (1928) reported loss of the satellite of the *tectorum* D chromosome in the F_1 *capillaris-tectorum* hybrid. This observation was confirmed by Hollingshead in the same hybrid (1930). In the F_1 *nicaeensis-setosa* hybrid the *nicaeensis* D chromosome is similarly modified and careful measurements indicated an increase in size of the proximal arm. This change makes it more difficult to distinguish between the B chromosome of *setosa* and the modified D chromosome of *nicaeensis*. The chromosomes of the five F_2 plants discussed in this paper are shown in figure 3*d*. In order to check the camera-lucida drawings, photomicrographs were made from normal somatic plates (plate 14*f*). The chromosomes appear to be as follows: from *setosa*, one A, two B, one C, and two D; and from *nicaeensis*, two modified D chromosomes. This genom, however, is outside of normal expectations and studies of meiotic behavior in both F_1 and F_2 indicate that one of the two chromosomes, which is morphologically similar to the *nicaeensis* D chromosome, is perhaps a modified *setosa* B chromosome. The evidence favorable to this hypothesis will be discussed under meiosis.

It is unusual that all five of these plants should have the same chromosome complement, and that no other combination of eight chromosomes was found. The eight F_2 plants that died in the seedling and very early rosette stages were most likely 8-chromosome types that were unable to survive. When the first lot of F_2 seed was planted, some care was exercised to eliminate the shrunken achenes. When a second planting was made, the achenes were carefully graded into large and small lots. There was a mortality of 40 per cent in the seedlings from the small achenes and of only 2 per cent in seedlings from large achenes.

Meiosis in F_1

Hybrid A (*C. nicaeensis* ♀ × *C. setosa typica* ♂)

This hybrid exhibited rather high regularity in its meiotic behavior. In figure 5 the drawings are from several slides, all stained with Haidenhain's, with the exception of *b*, *d*, and *h* which were stained in gentian violet. The number of bivalents ranged from one to four. Figure 5*a–d* shows associations of 4_{II}, $3_{II} + 2_{I}$, $2_{II} + 4_{I}$, and $1_{II} + 6_{I}$, respectively. The chromosomes in *b* and *c* are drawn out of position for the sake of clarity. Figure 5*e* shows four adjacent PMC's in the same locule, all exhibiting complete pairing. In three a small chromosome is loosely paired with a large one. The small chromosome is undoubtedly the *setosa* C chromosome. Figure 5*f* shows $3_{II} + 2_{I}$ at IM. The univalents were apparently

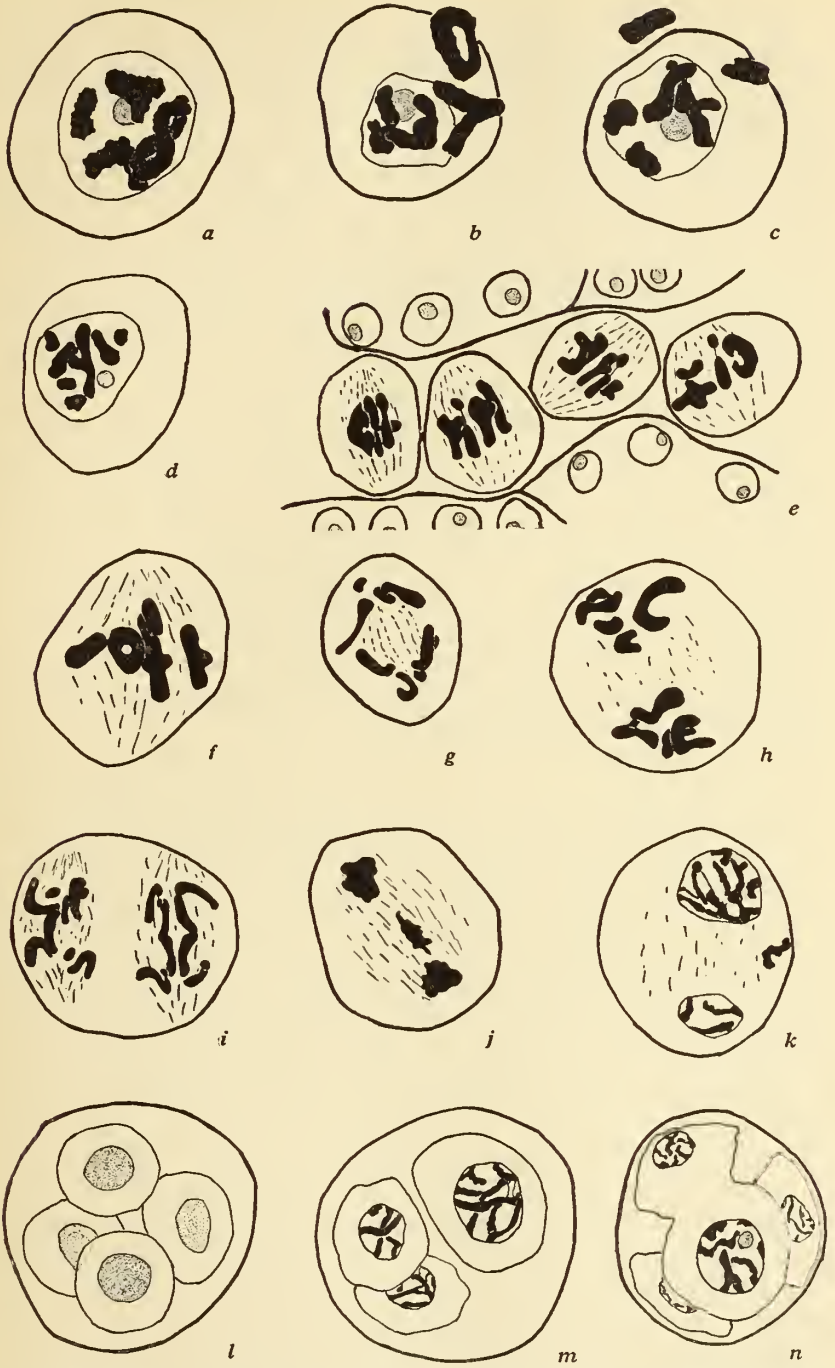

Fig. 5. Meiosis in hybrid A (*C. nicaeensis* × *C. setosa typica*). *a–d*, selected diakinesis figures showing variable pairing (in *b* and *c* certain chromosomes have been drawn outside the nucleus for clarity). *e*, adjacent PMC's showing complete pairing. *f*, IM—$3_{II} + 2_{I}$. *g*, IA. *h*, IIM. *i*, IIA. *j*, *k*, *m*, *n*, types of irregularities. *l*, "normal" tetrad. × 1875, except *e*, 1575.

not arranged on the spindle but were placed to the side of it. Their size indicates that neither is the *setosa* C chromosome. In ten cells showing $3_{II} + 2_{I}$, the C chromosome of *setosa* was one of the two unpaired chromosomes in only four cells.

Figure 5*g* shows a IA in which the *setosa* C chromosome is easily recognized. The chromosomes have not yet assumed their characteristic shapes, as is also true in the IIM shown at *h*, which may have followed such an anaphase. Figure 5*i* is an excellent IIA, showing the chromosomes in a nearly normal somatic condition. In the group to the right, the D chromosome of *setosa* can be recognized as well as its small C chromosome in the group at the left. Such a IIA would probably give rise to a tetrad similar to figure 5*l*. Very few irregularities were observed. Figure 5*j* shows a IT in which a portion of the chromatin has failed to reach either pole and will probably be left in the cytoplasm as a micronucleus. In figure 5*k* unequal nuclei are seen, with a chromatic body in the cytoplasm at the right. There was no trace of a membrane about this chromatic body. Possibly figure 5*m* represents a triad with a diploid and two haploid nuclei. It was the only example of this type found in the material examined. A type of tetrad irregularity that occurred very rarely is shown in figure 5*n*. There has been an unequal division of chromatin in the IIM of one nucleus of the dyad, whereas the other nucleus appears to have divided more regularly.

The frequencies of pairing in the two F_1 plants and in the F_2 plants are shown in table 3.

The large number of instances in which four bivalents occur is rather remarkable for *Crepis* hybrids involving different subgenera. The pairing is also rather close, and there is apparently a very intimate association of the chromosomes of most bivalents. The small C chromosome of *setosa* can frequently be identified, and it is usually very loosely paired with a *nicaeensis* chromosome. The generally close association of these chromosomes from widely separated species is quite different from the looseness of pairing reported in most of the other interspecific hybrids of *Crepis*. Avery (1930) published meiotic figures of hybrids involving *Crepis leontodontoides* with five other species—*tectorum, capillaris, parviflora, Marschallii,* and *aurea*. In many cells the bivalents are composed of rather loosely associated chromosomes which typically form elongated bivalents. The same situation also occurs generally in the hybrids studied by Babcock and J. Clausen (1929), who also published meiotic figures of *Crepis aspera* and *C. bursifolia*. The bivalents formed in these species show an association more nearly resembling the bivalents in our hybrid. Such close association in this hybrid indicates at least that conditions were rather favorable for exchange of material between chromosomes of the two species. This supposition is strengthened by the investi-

gation of meiosis in the hybrid *Crepis divaricata* × *C. Dioscoridis* by Müntzing (1934). Although the amount of pairing was noticeably lower than in our hybrids, the nature of the association was similar. Cross-shaped bivalents sometimes occurred, which would indicate interstitial chiasmata, and "the frequent occurrence of chromatin bridges and fragments at IA is too regular to be due simply to non-homologous association."

TABLE 3

BIVALENT FORMATION AND ACHENE PRODUCTION IN F_1 AND F_2

	4_{II}	$3_{II}2_I$	$2_{II}4_I$	$1_{II}6_I$	$0_{II}8_I$	Per cent irregularities	Per cent "good" pollen	Number of achenes
Hybrid A.	122	10	1	1	0	9	92	545
Hybrid B.	64	32	16	2	0	43	58	120
F_2 no. 1...	22	44	2	6	1	71	48	7
F_2 no. 2...	31	72	7	12	2	75	42	12

Hybrid B (*C. nicaeensis* ♀ × *C. setosa Topaliana* ♂)

In this hybrid the meiotic behavior of the chromosomes is considerably more irregular than in hybrid A. As shown in table 3, these irregularities comprise 43 per cent of the total, as compared with about 9 per cent for the same classes in hybrid A. In hybrid B, as well as in hybrid A, no cells were found with a complete failure of bivalent formation.

A diakinesis figure with 4_{II} is shown in figure 6*a*. In figure 6*b* there are $3_{II} + 2_I$; and the smallest univalent is very probably the C chromosome of *setosa*. Figure 6*c* represents three adjacent pollen mother cells with 4_{II}, $3_{II} + 2_I$, and $2_{II} + 4_I$ and shows the occurrence of loosely paired bivalents and the close association of variable pairing in the same locule. This figure may be compared with figure 5*c*, to show the looser type of pairing and relatively less frequent formation of 4_{II} in this hybrid than in hybrid A. In figure 6*d* the association of the small *setosa* C chromosome with a large *nicaeensis* chromosome forms a bivalent somewhat similar to one formed in hybrid A. One of the two cells observed, in which but one bivalent was formed, is shown in figure 6*e* in which the chromosomes were very loosely arranged near the equatorial plate. Figure 6*f, g* represent adjacent PMC's, one of which shows $2_{II} + 4_I$ and the other $3_{II} + 2_I$. In the second cell, two of the bivalents are very loosely associated, in striking contrast to the same situation in hybrid A (fig. 5*f*). In figure 6*h* one chromosome is seen lagging at the equator, four are moving to one pole, and three are going to the other. Three of the disjoined chromosomes already show equational splitting for the homoeotypic division. In figure 6*i* one chromosome is at one pole, five are at the

Fig. 6. Meiosis in hybrid B (*C. nicaeensis* × *C. setosa Topaliana*). *a*, *b*, diakinesis. *c*, adjacent PMC's showing variable pairing. *d–g*, metaphase plates showing rather loosely formed bivalents. *h*, *i*, IA showing unequal distribution of chromosomes. *j*, normal tetrad. *k*, dyad. *l–o*, variations found in same locules with "normal" types. × 1875, except *c*, 1575.

other pole, and two are lagging at the equator but appear to have begun migrating toward the pole with the larger chromosome number. An apparently normal tetrad is shown in figure 6*j*. Figure 6*k* represents a dyad in which the nuclei appear to be equal in size. Very few irregularities of this sort were seen in this plant, and where they did occur they were in locules in which tetrads occurred. Figure 6*l* shows a dyad in which the nuclei are unequal in size. This condition could have followed an unequal distribution of chromosomes like that shown in *h*. A normal monad was not found in this plant, but several similar to figure 6*m* were seen. Most of the tetrads were abnormal, micronuclei being very common.

Meiosis in F_2

The various stages in meiosis of the F_2 plants with 8 chromosomes are shown in figure 7. These drawings were made from smear preparations from the two plants which flowered. Diakinesis figures with 4_{II} and $3_{II} + 2_I$ are shown in figure 7*a, b*. Some IM figures are shown in figure 7*c–j* depicting 4_{II}, $3_{II} + 2_I$, $1_{II} + 6_I$, and 8_I. In figure 7*i* the C chromosome of *setosa* is a member of the only bivalent formed. Two IA groups are seen in figure 7*k, l*. In figure 7*l* some of the separated chromosomes are splitting for the homoeotypic division. An irregular assortment of 5 and 3 chromosomes is seen in figure 7*m*. A division of this type could give rise to very small pollen grains like those shown in figure 8*i*. The chromosome group in figure 7*n* is interesting because it suggests a possible method of origin for polyploid gametes. The seven units at one pole may have occurred by nonconjunction in three pairs of chromosomes. This situation was observed but once and cannot account for the high frequency of large pollen found in this plant. Many of the tetrads were normal (fig. 7*o*), but usually irregular types were found (fig. 7*p* and fig. 8*f, g, h*).

The frequency of the irregularities in meiosis in these two plants is in rather close agreement, as is shown in table 3. It is very unusual that in so many cells only one or two bivalents are formed. Unfortunately meiosis in the two subspecies has not been studied, so it is not known whether either of them shows an appreciable amount of nonconjunction.

Chromosome morphology would seem to indicate that these F_2 plants had six *setosa* chromosomes, one A, two B, one C, and two D, along with modified D chromosomes from *nicaeensis*. This complement, however, could be produced only if the *nicaeensis* D chromosome had paired part of the time with *setosa* C and part of the time with *setosa* A. This behavior would necessitate that the *nicaeensis* D chromosome have sectors, one homologous to a sector in the *setosa* C chromosome and another to a sector in the *setosa* A chromosome. If this were true, we should occasion-

Fig. 7. Meiosis in 8-chromosome F_2 plants. *a*, *b*, diakinesis showing 4_{II} and $3_{II}+2_I$; in *b* note the small unpaired chromosome (see text). *c-j*, IM showing variation in number of bivalents. *k-m*, IA (note small C chromosome of *setosa* in *l* and unequal distribution of chromosomes in *m*). *n*, seven chromatin bodies at one pole; possibly a result of attachment. *o*, *p*, "normal" and abnormal tetrads. × 1875.

ally find trivalents formed in these plants. As trivalents are not found this view is apparently not tenable.

The interpretation which seems most logical is that one of the two chromosomes which appear like *nicaeensis* D chromosomes is in reality a new chromosome resulting from crossing over in F_1. Although five F_2 plants had eight chromosomes that were apparently of the types described above, only two flowered normally and both were descendants of hybrid A. The pairing in this F_1 hybrid was exceedingly regular, and bivalent associations were in most cells very intimate. Hence exchange of material probably took place in this hybrid. If pairing occurred between *setosa* A and *nicaeensis* C, and crossing over took place at or near the constriction of *setosa* A, a new chromosome would be produced, the major portion of which would be *setosa* A, and the presence of the proximal arm of the *nicaeensis* C chromosome would produce an element resembling a *nicaeensis* D chromosome. On this basis the complement of these F_2 plants may consist of the following *setosa* chromosomes: one A, two B, one C, two D, and one modified A, together with one modified *nicaeensis* D chromosome. The haploid sets of *setosa* and *nicaeensis,* together with an F_2 somatic plate, are shown in figure 4. In the F_2 plant the chromosomes in question are marked X_1, X_2, and X_3.

The chromosomes of these F_2 plants may perhaps represent recombination products from crossing over in F_1. Although the chromosomes of each species are distinctively different in morphology, it would become exceedingly difficult to identify many possible new recombinations. Plants with other assortments of 8 chromosomes were unfortunately not found; they might have thrown considerable light on the question at issue. Navashin (1926) reported the appearance in *Crepis* of large atypical chromosomes, a phenomenon to which he gave the name "novation." Their occurrence was thought to result not from end-to-end fusion, but perhaps from translocation. Avery (1930) suggested that the variable pairing observed in the *Crepis* hybrids which she investigated "is a reflection of the transformational processes presumably responsible for the differentiation of the specific genoms." In a recent paper Navashin (1934) reports several occurrences of "sporadic amphiplasty" in which morphologically changed chromosomes were observed in the progeny of species hybrids. Poole (1932) has found cytological evidence of new chromosome types in derivatives of his *rubra-foetida* amphidiploid. In the hybrid, *Crepis divaricata* × *C. Dioscoridis,* Müntzing (1934) observed the occurrence at IA of chromatin bridges and fragments and concluded that the simultaneous production of long chromatids with two spindle-fiber attachments and fragments without attachment is probably caused by crossing over between homologous segments in chromosomes from the two species. He emphasizes the point that *"an unlim-*

ited number of new chromosome types may arise by the mechanism at work in this hybrid (or a number only limited by the number of chromomeres)."

Origin of Polyploid Gametes

When tetrad stages and pollen were studied in these F_2 plants, it was found that a comparatively large number of monads and dyads were formed and that the pollen grains could be sorted into four rather definite size groups, as shown in figure 8*j–l*. The group listed as "small" (table 4) and shown in figure 8*j* is probably of the "normal" size. On the basis of measurements of pollen grains in this group these grains were equivalent to "good" pollen in the parent species.

TABLE 4

Size of Pollen in F_2

Giant	Large	Small	Very small
35	78	115	16

In meiosis in F_1, and normally in F_2, whole chromosomes that were paired at IM separated and moved to the poles; and univalents were assorted at random without splitting. In the F_2 plants, IA figures were finally found in which splitting of both disjoined and univalent chromosomes occurred. Figures 8*a* and 8*b* indicate that the splitting has taken place slightly earlier in the disjoined chromosomes. In both chromosomes there is no doubt that the split had been completed in early anaphase and that the resulting nuclei would have had 8 chromosomes each. This type of behavior will probably result in a binucleate condition like that shown in figure 8*f*. The complexity of the chromosome situation in this F_2 plant makes it extremely probable that the resulting nuclei are very rarely alike qualitatively, even though each has 8 chromosomes. As the equational split has already occurred, it is unlikely that a second division of the chromosomes will occur; and various types of "diploid" gametes should result. Figure 8*k* is probably a "diploid" pollen grain resulting from such a division.

A second irregularity in meiosis was found which probably gives rise directly to the giant pollen grains that occur in these F_2 plants. Here, spindle formation at IM is very weak or entirely lacking. The chromosomes arrange themselves on the equatorial plate and some bivalents are probably formed, although this point is not certain and is not essential to what follows. Each of the 8 chromosomes splits equationally, but there is no separation of the halves; a nucleus of 16 chromosomes is the result. The 16 chromosomes can be counted readily in figure 8*e*. Various

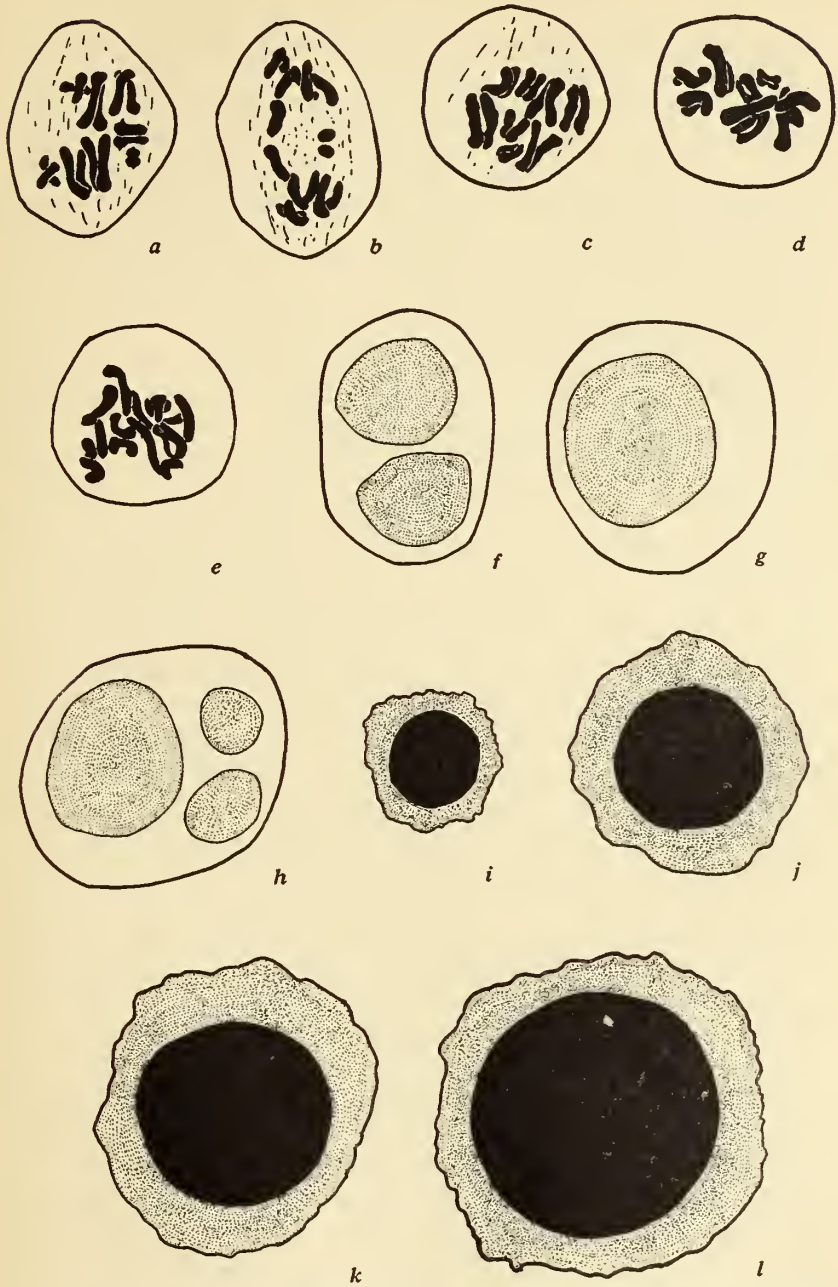

Fig. 8. Origin of polyploid gametes in an 8-chromosome F_2 plant. *a, b,* first anaphase showing precocious splitting of chromosomes which will probably result in "diploid" nuclei. *c–e,* origin of 16-chromosome gametes through splitting of the 8 univalents in equatorial region with no succeeding division. *f, g, h,* "dyad," monad, and "triad." *i–l,* pollen grains of four size classes, all × 1875.

stages of this type of division are shown in figure 8*c, d, e*. This condition would result directly in a monad (fig. 8*g*) which would develop into a giant pollen grain (fig. 8*l*).

Investigators working with widely scattered genera have reported the occurrence of giant pollen grains in many plants. De Mol (1923) found in Hyacinth, which had been subjected to abnormal conditions, large pollen grains in the nucleus of which was the diploid number of chromosomes. He interpreted their origin as due to an ineffective karyokinesis in the microspore rather than to any irregularities in the first or second division of the pollen mother cell.

Sakamura and Stow (1926) observed marked variation in the size of pollen grains in *Gagea lutea* from plants exposed to high temperatures. Cytological studies revealed several irregularities which they thought caused the abnormal pollen grains. In the first division they occasionally found an irregular distribution of chromosomes that resulted in pollen grains with varying chromosome numbers. Before the second division in some cells they observed that all chromosomes of the primary sporocyte gathered at the center of the cell and reconstructed a new nucleus without a cell division. They suggested that nuclei of this type probably gave rise to diploid pollen grains if a regular second division took place, or that, if this division were omitted, giant tetrad pollen grains would be formed. Second divisions were not observed.

Rosenberg (1926–27) investigated meiosis in a number of parthenogenetic species of *Hieracium*. He found a graded series of degeneration of meiosis ranging from the *boreale* type, in which a variable number of doubles and singles occur, to the *laevigatum* type, in which there is no conjugation whatever, namely, "semiheterotypic division." The latter type is often interrupted by a premature homoeotypic division whereby a "restitution nucleus" is formed. A new nuclear wall encloses the entire spindle figure and the chromosomes of this single large nucleus divide normally, producing pollen grains with the diploid number of chromosomes.

Karpechenko (1927), in tracing the origin of polyploid F_2 plants from his generic hybrid *Brassica* × *Raphanus*, found diploid pollen grains formed in the F_1 in the following manner. At IM pairing between the chromosomes of each haploid set did not occur and spindle formation was very weak with no distribution of univalents. The complete omission of the heterotypic division was followed by a normal homoeotypic division, each chromosome splitting equationally and separating to the poles. This resulted in diploid dyads. A second splitting of some univalents was occasionally observed. Tetraploid gametes were found to originate as follows: "In the cells of the archesporium, at the division preceding reduction division, a division of the nucleus sometimes occurs

without formation of a new cell wall. As a result there are produced pollen mother cells with two nuclei, each of them containing 18 chromosomes. The chromosomes in both nuclei remain nonconjugating; at the first division both spindles fuse so that the anaphase shows all the univalents which later on form one nucleus containing all the chromosomes. A splitting of all the chromosomes then ensues, and in the majority of cases dyads arise with about 36 chromosomes in each cell."

The two F_2 plants in which the polyploid gametes were observed were grown under separate environmental conditions. After the chromosome complements were determined, one plant was removed from Davis to Palo Alto and isolated. The second plant was isolated at Davis. Although Palo Alto and Davis are less than one hundred miles apart, there is quite a difference in the average temperatures of the two places. At Davis the temperature frequently reaches 100° F, while at Palo Alto it is usually not more than 80° to 85° F. The data on metaphase pairing in these two plants are given in table 3; number 1 was at Palo Alto, and number 2 at Davis. There is very little difference in the percentage of each type of pairing observed. This fact suggests that the occurrence of meiotic irregularities in these similar F_2 plants was not affected by such differences in temperature as were involved. It seems probable, therefore, that this temperature range (80°–100° F) may be included within a certain optimum range for meiotic regularity in these hybrids.

Later, the twelve achenes from F_2 plant number 2 were planted, and eleven seedlings were produced. At the same time, larger numbers of other *Crepis* seeds were planted, and all populations were transplanted into 4-inch pots in the same type of soil. The eleven seedlings from plant number 2 grew very rapidly and soon produced fine rosettes. They did not produce many roots, however, and finally the entire population became chlorotic and died. An examination of the plants a few days before death showed a very meager root system as compared to top growth. The other *Crepis* plants on the same bench grew vigorously. Only a few root tips were obtained from three of these F_3 plants and they were in poor condition. They showed that these three plants had 8 chromosomes; but the figures were so poor that identification was impossible. Definite conclusions concerning viability of the polyploid pollen cannot be drawn from such meager data, but Matsuda (1927) has shown that large pollen grains of *Petunia* are much less viable and slower to germinate than are normal ones.

Intraspecific Relations and Meiotic Behavior

The differences in amount of pairing at metaphase in hybrid A and hybrid B (table 3) show that the chromosomes of *C. setosa Topaliana* fail to form bivalents at metaphase with the *nicaeensis* chromosomes as

regularly as do the chromosomes of *C. setosa typica.* Also, when bivalents occur, the association of the paired chromosomes is generally less intimate in hybrid B than in hybrid A. That is, there are more bivalents in hybrid B showing merely terminal association. Such differences in meiotic behavior, however, do not necessarily indicate wide genic diversity between these two forms of *C. setosa,* because comparable differences have been found among closely similar individuals of a single species and in hybrids involving less diverse forms of a species.

Plants of a certain race of *C. capillaris* known as the X strain are morphologically typical of the species and closely similar to one another except for minor variations. Hollingshead (1930) found univalents at metaphase in from 12 to 44 per cent of PMC's in different plants of this strain growing under the same favorable conditions. More recently it has been discovered (Richardson, 1935) that, in progeny from a diploid branch of a haploid individual of this same strain of *C. capillaris,* the occurrence of univalents at IM is caused by failure of chiasma formation between homologous chromosomes *which are found to be regularly associated at pachytene.* She reports that such univalent homologous chromosomes frequently lie in juxtaposition at diakinesis and this is thought to be a result of their earlier association. Whatever the cause of failure in chiasma formation may be, the fact remains that marked irregularities in metaphase pairing may occur in similar individuals of the same species. It has been found by Beadle (1930) and others in several species of plants that such differences between individuals may be caused by a single gene difference.

Furthermore, Hollingshead (1930) studied the occurrence of irregularities in metaphase pairing in hybrids between *C. capillaris* ($n = 3$) and *C. tectorum* ($n = 4$). In these experiments the X strain of *C. capillaris* was crossed with two strains of *C. tectorum* (1498 and 1648). These two strains of *tectorum* were similar in most respects, but it was noted by Hollingshead that the 1498 strain was more uniform and had descended from one plant, whereas the 1648 strain was grown from wild seed and varied in leaf shape and time of maturity; also that 1498 came from the Copenhagen Botanic Garden, but that 1648 was collected near Tomsk, Siberia. Although the original source of the Copenhagen strain is not known, it is probable that the two strains represent widely separated geographic races. Furthermore, the achenes of these two strains are significantly different in width and possibly in length. At any rate, the Siberian strain has relatively narrow achenes. Such a difference is sufficient to warrant the recognition of these strains as distinct forms or perhaps as varieties, but they are certainly not sufficiently distinct in morphology to admit of their being recognized as subspecies. Yet the difference in meiotic irregularities in the hybrids between these two

strains of *tectorum* and the same strain of *capillaris* are even greater than the corresponding differences between the hybrids involving the two subspecies of *setosa*. This is shown in table 5, in which the original data are made more easily comparable by stating the percentages of PMC's showing the various numbers of pairs at metaphase. From these data it appears that hybrids 1A and 1B show less regular pairing at metaphase than do hybrids 2A and 2B. But this may be entirely due to the known tendency to meiotic irregularity in the *capillaris* X strain and not to a difference in the two *tectorum* strains. This may also account for the apparent tendency to greater difference in amount of meiotic

TABLE 5

COMPARISON OF MEIOTIC IRREGULARITIES IN CAPILLARIS-TECTORUM HYBRIDS AND IN NICAEENSIS-SETOSA HYBRIDS

Hybrids	Per cent of PMC's with				
	4II	3II	2II	1II	0II
1A. *capillaris* × *tectorum* 1498	..	55	36	8	1
1B. *capillaris* × *tectorum* 1648	..	18	30	25	27
2A. *nicaeensis* × *setosa Topaliana*	91	7	1	1	0
2B. *nicaeensis* × *setosa typica*	57	27	14	2	0

irregularity between 1A and 1B than between 2A and 2B. From these data, therefore, no conclusion can be drawn concerning the bearing of the amount of pairing at metaphase on the degree of genetic relationship between two subspecific entities.

Other differences were found, however, between the two subspecies of *C. setosa,* besides the difference in behavior of their chromosomes when associated with *nicaeensis* chromosomes in hybrids. The morphological differences already noted (table 1) justify their rank as subspecies, and the behavior of their chromosomes in the hybrids discussed above is consistent with this classification. Babcock and J. Clausen (1929) have shown that the closer the phylogenetic relationship between the species used in a cross, the higher the percentage of good pollen grains and the greater the fertility of the hybrid. The pollen counts of the hybrids, given in table 3, show a very striking difference. The percentage of meiotic irregularities observed in hybrid A, as compared with those in hybrid B, is of nearly the same ratio as the numbers of seed produced by each hybrid. Although their chromosomes correspond closely in size and shape, these subspecies of *setosa* may have had their chromosomal or-

ganization greatly altered. The fact that attempts to cross them were unsuccessful might indicate such a situation, but such negative results are of slight value in judging of relationships. The use of other forms of the same subspecies might possibly result in hybrids. If they do seldom cross in the regions where their ranges overlap, this would be an important factor in the divergence of the two entities. The point of importance to taxonomy is the fact that two entities exist which appear to have diverged from one species and which differ in several morphological details, but which are still so similar that practical considerations oppose their being recognized as distinct species. When such a situation is found, together with intergrading forms in the region of overlapping, the most satisfactory systematic disposition of these forms is their treatment as subspecies.

A REVIEW OF THE EVIDENCE ON MEIOSIS IN INTERSPECIFIC HYBRIDS OF CREPIS WITH REFERENCE TO INTRAGENERIC RELATIONS

The three major subdivisions of the genus, namely, *Catonia, Eucrepis,* and *Barkhausia,* may be considered as natural in the sense that, as a general rule, the species within each subgenus are more closely related to each other than they are to species in the other two subgenera. But, as has already been pointed out (Babcock, 1936), the very close relationship between the subgenera, as indicated by comparative morphology, makes it difficult to separate them by hard and fast lines. Thus certain species of *Eucrepis* resemble *Catonia* in their relatively unspecialized involucre, while several others approach *Barkhausia* in the structure of their fruits. Also, at least one species of *Eucrepis* shows certain affinity with species in both the other subgenera. This particular species, *C. leontodontoides,* was involved in the five interspecific hybrids studied by Avery (1930). Its relationships to the other species involved in these hybrids are considered below. The point to be emphasized here is that *C. leontodontoides* is a bridging species between *Catonia,* the most primitive, and *Barkhausia,* the most advanced, of the three subgenera. Concerning the genus as a whole, the evidence from chromosome number and morphology (Babcock and Cameron, 1934) and from geographic distribution (Babcock, 1936) also indicates the close interrelationship between the three subgenera and suggests a monophyletic origin for the genus, or at least that it is a natural group which arose from a common progenial stock.

The study of interspecific hybrids in *Crepis* has provided additional evidence bearing on phyletic relations within the genus. In the early work of Collins and Mann (1923) the first occurrence of autosyndesis was discovered in the hybrid *C. setosa* × *C. biennis.* As the 20 chromosomes of *biennis* formed 10 pairs at IM the 4 *setosa* chromosomes were distributed at random. Thus metaphase pairing furnished only negative evidence on the relationship between these species. Subsequent work on this hybrid led to the discovery that *C. biennis* is an octoploid species with a base number of 5, which was later confirmed by direct study of the somatic chromosomes (Babcock and Swezy, 1935). In another hybrid, *C. capillaris* × *C. setosa* (Collins and Mann, 1923), it was first reported that no pairing of the chromosomes was observed and that the fertility was extremely low. But it was reported later by Hollingshead (1930; based on unpublished data of Mann Lesley on a hybrid involving different strains of *capillaris* and *setosa*) that variable pairing was observed. It is possible that a small amount of metaphase pairing occurred

in the original hybrid of Collins and Mann. Yet another hybrid, *C. capillaris* × *C. aspera,* has been reported (Navashin, 1927) as very irregular in meiosis, with random distribution of unsplit or split univalents; but possibly a small amount of pairing also occurs in this hybrid. The occurrence of pairing in all other interspecific *Crepis* hybrids examined certainly warrants the recognition of this possibility.

Most of the other studies of meiosis in *Crepis* hybrids were made before the relations between pachytene chiasmata and pairing at metaphase had been worked out (Darlington, 1932) and the few attempts to study the premeiotic prophases in *Crepis* hybrids had proved unsuccessful. The recent contributions of Richardson (1935*a, b*) on meiosis in *Crepis* have shown that the striking individuality of the chromosomes and their meiotic behavior in species with low numbers offsets the technical difficulties involved in the small size of the florets and the paucity of pollen mother cells. Koller (1935) investigated meiosis in *Crepis aurea* ($n = 5$) of subgenus *Catonia* and *C. rubra* ($n = 5$) of subgenus *Barkhausia* and found that there is a definite difference with respect to the initial chiasma frequency and the degree of terminalization. It was concluded that the chromosome complements of two taxonomically well-differentiated species of the same genus may be similar in external structure, but dissimilar in behavior in meiosis, thus showing the important internal differences between these species.

Hitherto, the study of meiosis in species hybrids in *Crepis* has been restricted to the counting of bivalents and univalents appearing at metaphase. In these studies the proportion of bivalents observed in the various hybrids has been regarded as an indication of the degree of genetic affinity between the parent species. According to Darlington (*op. cit.,* pp. 159–160), amount of pairing between hybrids of various species cannot be compared as an indication of genetic similarity without a knowledge of chiasma frequencies in the parents; and differences in amount of pairing will have a very indirect relation to the differentiation in genetic properties of the parent species. Accurate data on chiasma frequency and behavior in parents and hybrids are no doubt essential for the elucidation of metaphase association in hybrids characterized by a mediocre or low amount of pairing. But if persistent chiasmata are essential for bivalent association at metaphase, then the amount of metaphase pairing must directly result from the number of such chiasmata. Also the abundance of evidence that pairing of chromosomes at pachytene is usually determined by the conjugation of homologous chromomeres justifies consideration of the occurrence of a number of bivalents at metaphase as direct evidence of genetic homology between the parent species. Moreover, when high and low amounts of pairing at metaphase are positively correlated with other evidence on genetic relationship,

these differences in pairing may be considered as consistent and cogent evidence on relationship of the parent species. With these considerations in mind, it seems worth while to reëxamine the data on chromosome pairing in all the interspecific hybrids of *Crepis* in which such studies have been made.

In order to compare the evidence thus far available from the study of these hybrids with the other categories of evidence already mentioned, comparable data on the meiotic behavior of the chromosomes in F_1 plants from eleven different interspecific crosses have been assembled in table 6. In connection therewith, references are given to the original papers. In this table the proportions of PMC's with various numbers of bivalents are expressed as percentages of the whole number of PMC's in which it was possible to make counts and, in order further to facilitate comparison of the hybrids, the mean numbers of bivalents per PMC are given. The classification of the species in subgenera is indicated typographically as follows: *Catonia,* capitals; *Eucrepis,* bold face; *Barkhausia,* italics. The phylogenetic relations of the species are roughly indicated by four categories in ascending order, viz.: primitive, less primitive, less advanced, advanced. It is unnecessary to go into detail here concerning the basis of classification into these categories. The features most used are life cycle, size of plant, shape of leaves, size of heads, size of outer involucral bracts, modification of inner bracts, size of florets, size and shape of fruits, number and size of chromosomes. It is believed that the classification here given is a fair representation of the relative phylogenetic position of the several species. Phyletic relations as based on morphology alone, however, are only approximate and the classification of the species into the four categories is more or less arbitrary.

Three of the hybrids, numbers 1, 9, and 11, are preëminent in the large amount of pairing exhibited. In each hybrid one of the parent species is more or less primitive and the other parent is in the next higher class. These facts are consistent with the idea that in more primitive species there have been relatively fewer genetic changes than in more advanced species. But hybrid 10 is like hybrids 9 and 11 with respect to phyletic classification of the parent species, and hybrid 10 shows a comparatively low amount of pairing. A brief consideration of the specific relations between the parents of each hybrid will throw light on this situation.

In hybrid 1, although *aurea* is of *Catonia* and *leontodontoides* of *Eucrepis,* both species are perennials, and they are rather similar in certain morphological features, particularly in size and shape of the fruits. Also they both have 5 pairs of chromosomes which are closely similar in size and shape.[1] Furthermore, they are indigenous in the same

[1] All comparisons of karyotypes are based on Babcock and Cameron (1934).

TABLE 6

COMPARISON OF CHROMOSOME PAIRING AT METAPHASE IN ELEVEN INTERSPECIFIC *Crepis* HYBRIDS WITH REFERENCE TO SUBGENERIC CLASSIFICATION AND RELATIVE PHYLETIC STATUS OF THE SPECIES INVOLVED

(Classification of species into subgenera indicated by capitals (*Catonia*), boldface (*Eucrepis*), or italics (*Barkhausia*). Haploid chromosome numbers stated.)

No.*	Primitive	Less primitive	Less advanced	Advanced	Per cent of PMC's with						Total PMC's	Mean number of bivalents per PMC
					5II	4II	3II	2II	1II	0II		
1	AUREA 5	**leontodontoides 5**			74	23	3	0	0	0	53	4.7±.16†
2		**leontodontoides 5**	**tectorum 4**		..	20	15	26	19	20	54	2.0±.26
3		**leontodontoides 5**	**parviflora 4**		..	42	15	20	.04	22	27	2.5±.47
4		**leontodontoides 5**	**capillaris 3**		..	..	20	20	16	44	25	1.5±.25
5		**leontodontoides 5**		*Marschallii* 4	..	9	22	23	16	30	56	1.6±.22
6			**capillaris 3** **tectorum 4**		.. ..	A B	55 18	36 30	8 25	1 27	442 576	2.5±.09 1.4±.05
7			**tectorum 4**	*taraxacifolia* 4	..	30	27	37	6	0	30	2.8±.27
8			*aspera* 4	*bursifolia* 4	..	19	40	19	14	8	59	2.5±.28
9		*aculeata* 4	*aspera* 4		..	61	31	8	0	0	36	3.5±.18
10		*divaricata* 4	**Dioscoridis 4**		..	10	14	30	37	9	57	1.8±.23
11		**nicaeensis 4**	*setosa* 4		A B	91 57	7 27	1 14	1 2	0 0	134 114	3.9±.21 3.4±.18

* Basic data for numbers 1 to 5 from Avery (1930) and for number 3 additional unpublished data; for number 6 from Hollingshead (1930); for numbers 7 to 9 from Babcock and Clausen (1929); and for number 10 from Müntzing (1934).

† Standard error.

geographic area, although *aurea* is a montane species and *leontodontoides* occurs at low altitudes. This evidence, together with the fact of high degree of pairing in the hybrid, warrants the inference that although *leontodontoides* has developed the more specialized involucre of *Eucrepis* and in other respects is a less primitive species than *aurea,* yet there is a high degree of homology between the chromosomes of the two species.

The parents of hybrid 9, *aculeata* and *aspera,* are in the same section of *Barkhausia* and are obviously closely related; but *aculeata* is somewhat more primitive, having larger heads, florets, and fruits, less differentiated marginal achenes, and similar but much larger chromosomes. Therefore a high degree of genic homology may be assumed, and an even larger amount of pairing at metaphase than was observed might be expected. As 61 per cent of the PMC's had 4_{II} and 31 per cent 3_{II}, it is possible that the difference in size or some other structural difference prevented chiasma formation or terminalization in one particular chromosome type. A study of pachytene and later stages in this hybrid would be of interest. The two species occur in the same geographic area.

Hybrid 11 is the subject of the first part of the present paper, and the differences between 11A and 11B in number of pairs at metaphase have been discussed. At this point only hybrid A will be considered. Although *nicaeensis* is of *Eucrepis* and *setosa* of *Barkhausia,* and although *nicaeensis* is somewhat more primitive, being biennial, larger throughout, and with thicker, beakless fruits, nevertheless the two species are generally similar. This is true especially when *setosa typica* is compared with *nicaeensis,* and when habit, type of involucre, and size of heads, florets, and fruits are considered. The chromosomes are of the same four types, and in only one of the types do the representatives of the two species differ greatly in size. Furthermore, *setosa typica* is indigenous in low altitudes of the same geographic area as *nicaeensis,* which is montane. Hence the nearly complete metaphase pairing and the closeness of the association in the bivalents can be interpreted as indicating a high degree of genic homology in these species.

In hybrid 10 a quite different situation exists. The phyletic relation is the reverse of that in number 11, the *Barkhausia* species, *divaricata,* being somewhat more primitive than the *Eucrepis* species, *Dioscoridis.* Both species have 4 pairs of chromosomes. But on the basis of comparative morphology they are widely separated; for they differ throughout and notably in that *divaricata* has less reduced outer involucral bracts and monomorphic, shortly beaked achenes; whereas *Dioscoridis* has a rather highly specialized involucre and dimorphic, unbeaked achenes. The two species are widely separated geographically, *divaricata* being endemic in Madeira, and *Dioscoridis* in Greece. The amount of chromo-

some pairing in this hybrid is practically the same as in hybrid 5. In the latter the parents were *leontodontoides,* a relatively primitive species of *Eucrepis* with 5 pairs of chromosomes, and *Marschallii,* an advanced species of *Barkhausia* with 4 pairs. These species are indigenous in widely separated regions, *leontodontoides* in Italy and southwestern France, *Marschallii* in the Caucasus and Caspian region. All this evidence indicates that in both of these hybrids the parents represent widely divergent phyletic lines within the genus and is consistent with the notion that the low amount of metaphase pairing in these two hybrids indicates relatively low amounts of genic homology.

Hybrids 2, 3, and 4 may be considered together since the phyletic relations are similar. Unfortunately the data on numbers 3 and 4 are very limited and in number 3 the distribution of PMC's according to number of bivalents present is irregular. In all three hybrids both parents are *Eucrepis* species, and the more primitive one is *leontodontoides,* which is indigenous in Italy and southwestern France. The natural distribution of the other three species is as follows: *tectorum,* middle and northern Eurasia; *parviflora,* eastern Mediterranean to Caucasus; *capillaris,* southern and middle Europe east to Crimea. The mean numbers of bivalents per PMC are: hybrid 2, 2.0; hybrid 3, 2.5; hybrid 4, 1.5. The amount of pairing in number 2 is consistent with the view that, in *Crepis,* species which are widely separated in geographic distribution have less genetic homology than species occupying the same region. The slightly larger amount of pairing in hybrid 3, of which the parents are more widely separated geographically, is in apparent disagreement with this conception, but the small number of PMC's counted and their irregular distribution according to number of bivalents present, make this apparent exception less significant. Hybrid 4 also appears to be an exception to the hypothesis because the geographic areas of the parents are the closest in this hybrid, yet the amount of pairing is the lowest. But such an inference is not warranted for this hybrid, because the *capillaris* plants used as parents were all of the X strain in which wide variations in pairing occur between individuals

Hybrids 6, 7, and 8 may also be considered together. Although in number 6 two forms of *tectorum* were used, the differences in pairing observed were not comparable to those obtained in hybrid 11 because of the known variability of pairing in the *capillaris* plants used in this cross. For the same reason hybrid 6 cannot be compared with hybrids 7 and 8 with any degree of assurance. It may be noted, however, that the amount of pairing in number 6A is more nearly what might be expected from the morphological similarity of the two species and their geographic distribution. Hybrids 7 and 8 can be compared with more assurance. All four parental species have 4 pairs of chromosomes. Although number 7

was a hybrid between a *Eucrepis* species and a *Barkhausia* species and number 8 involved two *Barkhausia* species, the phyletic relations between the two pairs of species are similar in that one species is less advanced than the other. The two hybrids differ, however, in the degree of difference in advancement of the parents. In other words, the degree of advancement of *taraxacifolia* over *tectorum* is greater than that of *bursifolia* over *aspera*. But the amount of pairing in the two hybrids is equal, the slight difference between them not being significant. This apparent inconsistency is offset by the evidence from geographic distribution. In number 7 the areas occupied by the two species overlap, but in number 8 one parent (*aspera*) occurs in Asia Minor and the other (*bursifolia*) is endemic in Italy. Therefore the geographic distribution of the two pairs of species seems to correspond with their degree of genic homology, as indicated by metaphase pairing in their respective hybrids, if allowance be made for the difference in phyletic relations.

In the foregoing review the degree of genetic homology between pairs of species, as indicated by the number of bivalents at IM in their respective hybrids, has been considered with reference to the relative phyletic status of the species and their geographic distribution. The hybrids investigated are too few to warrant any conclusions, and the crossings were not planned with reference to a study of the relation of metaphase pairing to either phyletic status or geographic distribution considered alone. But, if both these variables are taken into account when the metaphase pairing in two hybrids is compared, two inferences appear to be indicated, as follows: (1) hybrids between more primitive species have larger amounts of metaphase pairing than hybrids between more advanced species; (2) hybrids between species occupying the same geographic region also exhibit greater meiotic regularity than do hybrids between species from widely separated regions. It is to be emphasized that these inferences apply only to the *Crepis* hybrids herein considered.

That hybrids between more primitive species of a monophyletic genus will exhibit greater meiotic regularity than do hybrids between more advanced species, is a reasonable *a priori* assumption, because the more primitive species would be less differentiated genetically in a truly natural group. From a study of meiotic behavior in *Nicotiana* interspecific hybrids, Goodspeed (1934) reached the conclusion that formation of bivalents in a hybrid is positive evidence that the pairing chromosomes possess many similar or equivalent genic elements; and that decrease in amount of pairing "is to be related to an accumulation of genic and chromosomal distinctions between species evolved from a common stock." The data on *Crepis* hybrids are not adequate for any generalization about phyletic status and meiotic regularity. There should be several series like hybrids 1–5, all of which involve a single species for one

parent, and both parents should occur in the same region. Even in this series numbers 3 and 4 must be disregarded because of unreliability of the data, and in number 5 the factor of wide geographic separation is introduced. This leaves only the first two hybrids, in which there is a significant difference in amount of pairing and a great difference in phyletic status of *aurea* and *tectorum*. The more primitive *aurea* produced a hybrid with high meiotic regularity and high fertility, and the more advanced *tectorum* gave a hybrid with low metaphase pairing and extremely low fertility.

With reference to the second inference, it seems probable that, in a recently evolved genus with many closely related but widely separated species, there is positive correlation between wide geographic distribution and a low degree of genetic homology. This also is a reasonable assumption because groups of most closely related species would be distributed from the center or origin during the course of evolution. In the present study hybrids 8 and 9 support this inference because all four species are of subgenus *Barkhausia,* the parents of hybrid 8 being widely separated geographically and less similar morphologically, whereas the parents of hybrid 9 occur in the same region and are generally similar. Hybrid 8 has a mean of 2.5 $\pm$ .28 bivalents per PMC, and hybrid 9 has a mean of 3.5 $\pm$.18. The difference, 1.0 $\pm$ .33, is three times its standard error and hence may be considered significant. A similar situation holds in hybrids 10 and 11 and in these there is a much greater difference in the number of bivalents formed in the hybrids. It should be emphasized, however, that this positive correlation between wide geographic separation and relatively low genetic homology is suggested only for the genus *Crepis* and, perhaps, for other large, recently evolved, but widely distributed genera.

As a matter of fact, exactly the opposite relation has been reported in certain genera, such as *Philadelphus* (Bangham, 1929; Sax, 1931) and *Platanus* (Sax, 1933). Both of these are old genera. According to Seward (1931), *Platanus* is one of the oldest of the broad-leaved trees, and we are informed by Chaney (*in litt.*) that there is no question of its presence in early Tertiary rocks in many parts of the world, including western North America. With respect to *Philadelphus,* Chaney (1927) has recognized it in middle Tertiary deposits of eastern Oregon, and Cockerell (1908) has recognized it in the Upper Miocene of Colorado. At all events *Platanus* and *Philadelphus* are certainly more primitive and certainly much older than *Crepis.* In both those genera hybrids have been obtained between certain Old World and New World species having the same chromosome number, and some of these hybrids exhibit complete or nearly complete pairing of the chromosomes, although there is much variation among them with respect to fertility. The perfect or

nearly perfect pairing at diakinesis or IM in these hybrids indicates a high degree of genetic homology in the parental species. Apparently the chromosomes of these widely separated species have undergone insufficient change to prevent pairing although the species have been isolated for millions of years. In the present discussion it is unnecessary to consider the problem of stability and plasticity in species and genera. It is sufficient to note that such differences exist and that relations between geographic distribution and genetic homology of species will vary accordingly.

Summarizing the evidence on intrageneric relations in *Crepis* leads to certain generalizations about this genus. *Crepis* is a large group of closely related species. Although natural major subgeneric groups are clearly indicated and these groups provide a convenient basis for systematic classification, yet the species thus classified under different subgenera are still more or less closely related. That is, their genic complements are more or less homologous. This is certainly indicated by the evidence on metaphase pairing in the interspecific hybrids reviewed above. It is supported also by two other categories of evidence. (1) It has already been shown (Babcock and Cameron, 1934; Babcock, 1936) that in several groups of morphologically similar species there is a single karyotype even though the species occupy different geographic areas. Evidently differentiation and distribution has not been accompanied in these groups by visible marked changes in the chromosomes. Thus the inference seems warranted that speciation has been made possible by a limited number of gene mutations or minute structural changes, leaving the main part of the residual genotype unchanged. (2) This inference is shown to be valid by the results of the investigations on three such groups of species made by Jenkins, Cave, and Smith independently (unpublished data). In each group the interspecific hybrids studied exhibited almost complete pairing at metaphase; and genetic experiments with alternative characters in different species proved that these characters are inherited in Mendelian fashion. In general, therefore, the evidence on metaphase pairing in interspecific hybrids in *Crepis* is consistent with all the other evidence on phylogeny in this genus.

SUMMARY AND CONCLUSIONS

1. Two subspecies of *Crepis setosa,* namely, *typica* from western Europe and *Topaliana* from Greece, were used in this study. They had been ranked as subspecies because of the differences they exhibited in external morphology. Each subspecies was used as the paternal parent in a cross made with the same plant of *C. nicaeensis.* The diploid chromosome number in both species is 8. The morphology of the chromosomes of the *setosa* subspecies is apparently the same, and they differ sufficiently from the members of the *nicaeensis* genom to make identification in F_1 relatively easy.

2. The interspecific hybrids were produced with difficulty, and only one plant from each cross was available for cytological study. These are designated as hybrid A (*nicaeensis* ♀ × *setosa typica* ♂) and hybrid B (*nicaeensis* ♀ × *setosa Topaliana* ♂).

3. The satellite of the *nicaeensis* D chromosome was not found in either F_1 plant, and in each plant there was an increase in the size of the head of this chromosome.

4. Meiosis in the F_1 plants showed that the chromosomes of *setosa typica* paired very regularly with the *nicaeensis* chromosomes and exhibited a very close association of chromosomes in bivalent formation, whereas those of *setosa Topaliana* exhibited less regular pairing and looser association. Percentage of good pollen grains was determined and showed a high correlation with meiotic behavior, being much greater in hybrid A than in hybrid B. Comparative fertility under open pollination was consistent with the foregoing evidence.

5. An F_2 population of 87 plants was grown from hybrid A, and one of 17 plants from hybrid B. In the first group, eighty-four plants were found to contain 24 chromosomes and three plants had 8 chromosomes; in the second group, fifteen plants had 24 chromosomes and the remaining two plants had 8 chromosomes. The 24-chromosome plants were found to have arisen by spontaneous crossing with *Crepis biennis.* With the exception of but six plants, they were all biennial. Each of the six that bloomed the first year was slightly fertile under isolated conditions.

6. The F_2 plants with 8 chromosomes were studied carefully because of their unusual chromosome complement. The morphology of these 8 chromosomes indicated that 6 were from *setosa*—one A, two B, one C, and two D; and the two remaining were apparently *nicaeensis* D chromosomes. This supposition, however, was not supported by meiotic behavior, either in F_1 or in F_2; and it was suggested that an exchange of material occurred in F_1 and that the F_2 chromosomes represent recombination products of this exchange.

7. The various stages in the formation of giant pollen grains in F_2 plants with 8 chromosomes were observed. But polyploids were not obtained in F_3, primarily because of high sterility of the F_2 plants and low viability of the F_3 progeny. Slower germination of the polyploid pollen may have been a secondary factor.

8. The two subspecies of *Crepis setosa* are so similar morphologically that their classification as different species does not seem to be justified. Although their chromosomes are exactly similar in appearance, yet they must differ somewhat in genetic composition. This is indicated both by the morphological differences between the subspecies, and by the differences in metaphase pairing in hybrids A and B. Classification as subspecies, at least for the present, has the advantage of emphasizing their very close affinity.

9. The genus *Crepis* is a group of closely related species. The subgenera, *Catonia, Eucrepis,* and *Barkhausia,* are not widely differentiated, but overlap more or less, and several hybrids have been produced between species of different subgenera. Eleven interspecific hybrids between diploid species have been studied by various investigators with particular reference to the amount of pairing at metaphase. The evidence indicates that the genic complements of the fourteen species involved are all more or less homologous. This inference is consistent with the evidence on chromosome morphology in the genus, on geographic distribution, and on other results (as yet unpublished) from genetic investigations on groups of closely related species. The evidence on metaphase pairing in interspecific hybrids, therefore, supports the conception that the subgenera of *Crepis* had a common origin and are still more or less similar in genetic composition.

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PLATE 14

Fruiting heads

a. Crepis setosa typica (2623).

b. C. setosa Topaliana (2671).

c. C. nicaeensis (2700).

d. C. nicaeensis ♀ × *C. setosa typica* ♂ F_1 (30.31).

e. C. nicaeensis ♀ × *C. setosa Topaliana* ♂ F_1 (30.32).

Somatic chromosomes

f. Photomicrograph of mitotic metaphase in a root-tip cell of an 8-chromosome F_2 plant.

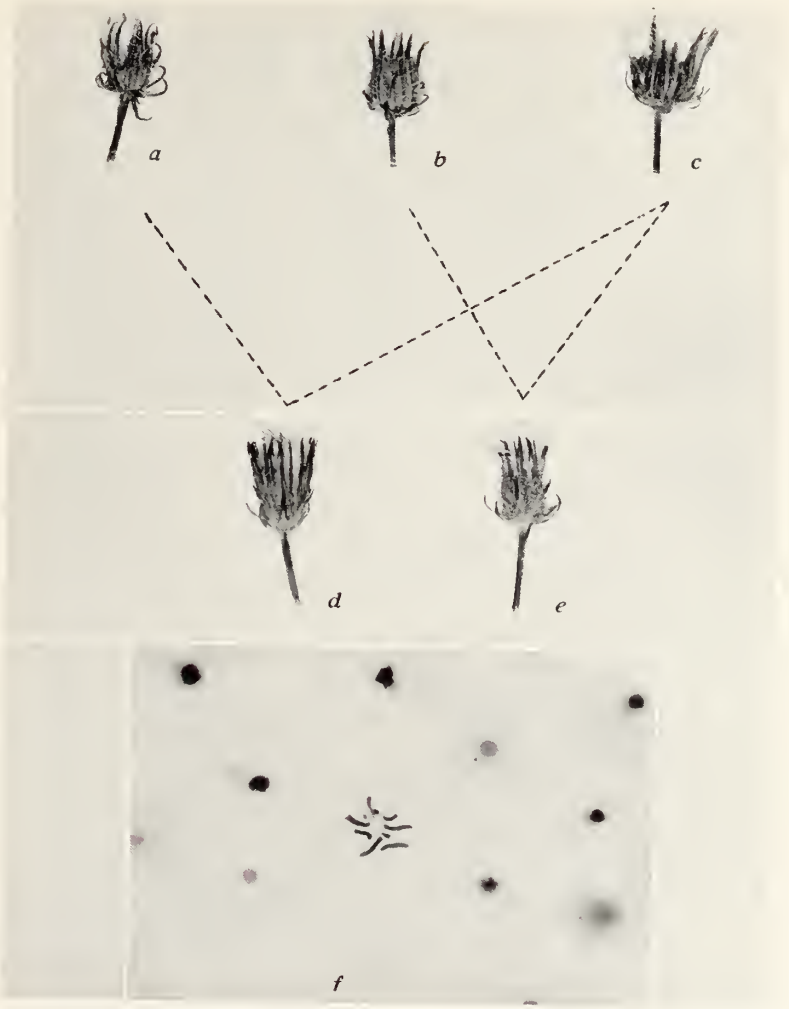
a
b
c
d
e
f

PLATE 15

(*Crepis nicaeensis* × *C. setosa typica*) ♀ × *C. biennis* ♂

a–e. Basal leaves from five 24-chromosome plants derived from open-pollinated F_1.

Crepis nicaeensis × *C. setosa typica*, F_2 progeny.

f, *g*. Two of the 8-chromosome plants.

h, *i*. Two of the plants that died in early rosette stage, before the chromosomes were examined.

a
b
c
d
e
f
g
h
i